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THE RESPONSE OF SODIUM OF BONE TO ACUTE HYPONATREMIA

Muriel Ruth DuBrow

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THE RESPONSE OF SODIUM OF BONE

TO ACUTE HYPONATREMIA

by

Muriel Ruth DuBrow Wolf
Smith College, B.A., 1957

A Thesis Presented to the
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INTRODUCTION

It is well known that skeletal tissues are rich in sodium. Sixty-five years ago Gabriel[1] showed that substantial amounts of sodium existed in the chloride-free residue of bone extracted with alkaline glycol solution. Harrison, Darrow, and Yannet[2] showed in dogs that the distribution of body sodium is closely related to body chloride if sodium in the skeleton is excluded. In the skeleton the amount of sodium is greatly in excess of the chloride; this was called the "extra" bone sodium. They found in dogs that approximately 50% of the body sodium is found in cartilage and bone, and about half of this bone sodium is "extra" bone sodium. Other investigators[3,4] have found that one third of the total body sodium is found in the skeleton. Forbes and Perley,[3] Kaltreider et al.,[4] and Edelman et al.,[5] have shown that approximately 40% of bone sodium is exchangeable with radioactive sodium in the serum. The data of Edelman et al.[6] shows that greater than 50% of sodium associated with the solid phase of bone is excluded from direct contact with serum sodium, whereas almost all the bone water sodium is exchangeable.

The nature of the sodium in the "extra" bone sodium has been the subject of study of several investigators. Neuman et al.[7] interpreted their data as showing that the sodium is bound to the bicarbonate complex of the apatite structure of bone ($Ca_9(PO_4)_6 \cdot CaX_2$ where X is a bicarbonate), and they further postulated that in acidosis, by ionization, the sodium ion, and thus the sodium is released to the body. Bergstrom[8] has confirmed this work, and Bergstrom and Wallace[9] have suggested that the crystalline lattice containing sodium ions may act as an ionic exchanging

area capable of donating sodium to the body.

Previous studies by a number of investigators have shown that metabolic acidosis with hyponatremia will cause a movement of sodium out of the bone to help buffer the mineral acids[9-14]. The studies of Woodbury[15] in rats have been interpreted to show that acute hyponatremia per se will cause a depletion of bone sodium. The experiments of Nichols and Nichols[16] in dogs have been interpreted in a similar manner.

The purpose of these experiments was to explore further the relationship of hyponatremia to the sodium of bone.

METHODS

Experiments were performed on twenty-eight Sprague-Dawley albino male rats. During the experimental period the animals were not given food nor water.

Group A - ten animals were maintained throughout the experimental period of four hours without food or water; they were otherwise untreated.

Group B - nine animals were injected intraperitoneally with 5.5% glucose (10 c.c./100 grams body weight). The purpose of the experiment was to produce hyponatremia.

Group C - five animals were injected intraperitoneally with 5.5% glucose with 25 meq./L.of $NaHCO_3$ (10 c.c./100 grams body weight). The purpose of the experiment was to produce hyponatremia without acidosis.

Group D - four animals were injected intraperitoneally with 5.5% glucose with 25 meq./L.of NaCl (10 c.c./100 grams body weight).

Four hours after the intraperitoneal injections in Group B, C, and D, and four hours after Group A animals were fasted, the animals were anesthetized lightly with phenobarbital and sacrificed in room air.

A "T"-shaped incision was made on the abdominal wall and the animals were exsanguinated through the abdominal aorta using a heparinized syringe containing mineral oil. The procedure was terminated when the blood stopped flowing smoothly into the syringe.

A vertical incision was made bilaterally on the hind limbs, and the femurs were scraped clean of muscle and tendon and removed. The heads and distal epiphyses were cut off, and the marrow was removed from the shafts of the bones by repeated insertions of a #18 needle into the

marrow cavity. The two shafts were placed into a weighing bottle which was previously dried for 24 hours in an oven at approximately 100° C. and then weighed.

The wet bone specimens were dried for five days in an oven maintained at approximately 100° C. The specimens were then reweighed and ground to a fine powder using separate mortars and pestles. The tissue was redried for 24 hours and then reweighed, the drying and reweighing repeated until constant weights were maintained. The total weight lost was considered to be the water content of the tissues.

200 mg. specimens of powdered bone were placed individually in a 50 c.c. Erlenmeyer flask to which was added 4 c.c. of concentrated nitric acid. The tissues were digested overnight on a steam bath at approximately 90 to 100° C. After digestion the specimens were quantitatively transferred through glass wool to a 25 c.c. volumetric flask. The glass wool had been thoroughly rinsed in distilled water before using. The transferred specimen was diluted with distilled water to the 25 c.c. mark on the flask. The resulting diluted sample was used for sodium determinations.

The sodium concentrations were determined in duplicate on diluted aliquots by indirect flame photometry with the Baird Associates flame photometer.

Studies on the Blood

The pH was determined anaerobically on whole blood with a Cambridge Electron-ray pH meter.

Whole blood was transferred under oil to plastic test tubes and then centrifuged to separate the cells from the plasma. The plasma was transferred under oil through an oil containing syringe to a small vessel.

The total CO_2 of the plasma was determined on the Natelson microgasometer using the factors of Peters and Van Slyke.[17]

The plasma sodium was determined in duplicate on the Baird Associates flame photometer by indirect flame photometry.

RESULTS

The results of all the experiments are listed in Table I.

Peritoneal infusion with 5.5.% glucose produced a depression of plasma sodium from the untreated level of 152.7 meq./L. to 131.8 meq./L., and a depression of the plasma CO_2 from the untreated level of 26.69 mM./L. to 19.47 mM./L. This was associated with a fall in bone Na from the untreated level of 0.2743 meq./gram wet bone to 0. 2636 meq./gram wet bone. See Table II. In rats of group C (Table V) an attempt was made to maintain the plasma CO_2 at the untreated level, but to lower the plasma sodium, by infusing 5.5% glucose with 25 meq./L. of $NaHCO_3$. This resulted in a significant decrease of the plasma Na(128 meq./L.) to approximately the same level as animals treated with glucose alone (131.8 meq./L.). Plasma CO_2 was slightly lower in glucose and $NaHCO_3$ treated animals (24.8 mM./L.) as compared to 26.69 mM./L. in control animals, but this is probably not physiologically significant. The CO_2 of Group B (glucose treated animals) is much lower than that of animals treated with glucose and $NaHCO_3$.

The bone Na of the animals treated with glucose and $NaHCO_3$ (0.2534 meq./gram wet bone) was lower than that of control animals (0.2743 meq./ gram wet bone), but was not statistically different from the bone Na of animals treated with glucose alone (0.2636 meq./gram wet bone).

Thus Na depletion was associated with a significant drop in bone Na whether or not acidosis was also produced. There was no significant difference in the degree of hyponatremia or in the magnitude of the fall of bone Na in groups depleted of Na with 5.5% glucose alone (Group B), in the group in which $NaHCO_3$ was added to the peritoneal wash (Group C), and in the group of rats in which 25 meq./L. of NaCl was added to the peritoneal wash (Group D). See Table I, V.

To summarize these results graphically:

	Plasma Na	Plasma CO_2	Bone Na
Untreated Group A	Normal	Normal	Normal
Glucose treated Group B	Decrease	Decrease	Decrease
Glucose and $NaHCO_3$ treated Group C	Decrease	Normal	Decrease
Glucose and NaCl treated Group D	Decrease	Decrease	Decrease

By the method of multiple regressions, using the data from the untreated animals and also all of the data from the treated animals (glucose animals, glucose and $NaHCO_3$ animals, and glucose and NaCl animals), it is possible to derive an equation for the bone Na dependent upon the plasma Na. See Table VI. The following discussion will be concerned with the derivation of this equation relating plasma Na and bone Na.

When X_1 = plasma Na, when X_2 = plasma CO_2, and when Y = Bone Na, by using multiple regressions, it is possible to derive an estimate of the bone sodium. This Y estimated or bone sodium estimated will be referred to by the symbol $\hat{Y}$.

$$\hat{Y} = 0.000702\, X_1 - 0.0000134\, X_2 + 0.166749$$

When the significance of the two regression slopes is tested, that for plasma Na (X_1) is highly significant as the t value is larger than that for p = 0.01, whereas the slope for plasma CO_2 (X_2) is not significant (t is smaller than the t for p = 0.5).

The slope for X_1 is referred to in the data under Table VI as b'y1.2. The estimated value for the slope for X_1 is 0.604101. The slope of X_2 is referred to as $b'_{y2.1}$. The value is - 0.038946.

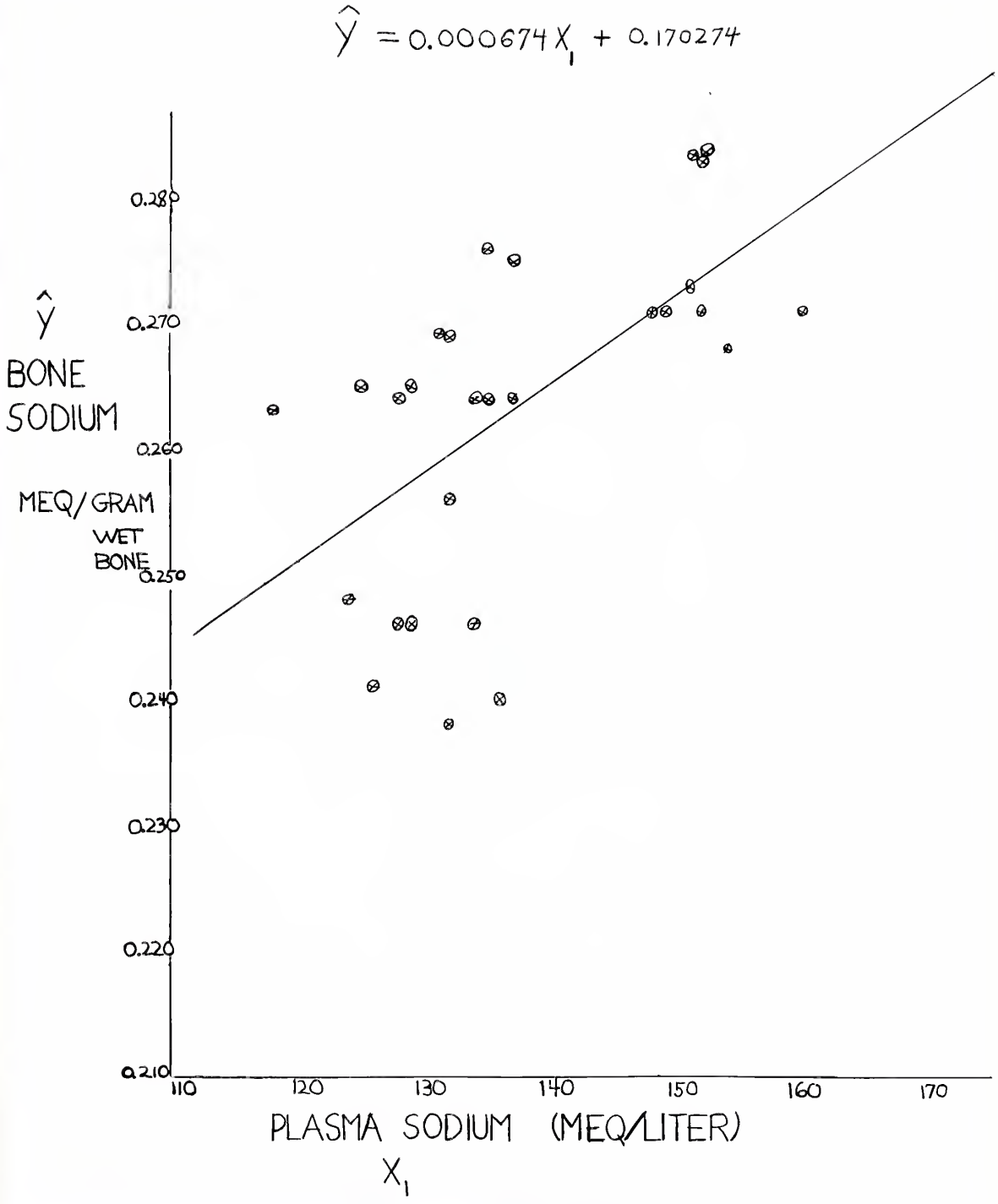
Therefore since the slope for plasma CO_2 is not significant, the plasma CO_2 may be disregarded as an important factor in the determinant of the level of bone Na; and a simple regression equation may be written as follows:

$$\hat{Y} = 0.000674\ X_1 + 0.170274$$

where $\hat{Y}$ = the estimate of bone Na and X_1 is the plasma sodium.

This equation gives the line of best fit of all the data presented here. See Figure I.

In untreated animals there was no significant difference in bone Na between animals weighing less than 250 grams (average weight of 226 grams) and animals weighing more than 250 grams (average weight of 305 grams). Similarly small rats (average weight of 219 grams) did not appear to lose more bone Na than larger rats (average weight of 298 grams) in response to Na depletion. Table IV.


$\hat{y} = 0.000674 X_1 + 0.170274$
$\hat{y}$
BONE SODIUM
MEQ/GRAM WET BONE
0.280
0.270
0.260
0.250
0.240
0.230
0.220
0.210
110
120
130
140
150
160
170
PLASMA SODIUM (MEQ/LITER)
X_1

DISCUSSION

In 1935 Darrow and Yannet[18] showed that when glucose was infused intraperitoneally and then removed, the plasma sodium was lowered.

Woodbury[15] in 1956 produced hyponatremia in rats by the method of Darrow and Yannet and then measured the bone sodium. He concluded that the bone sodium level fell with hyponatremia. However, he did not measure the CO_2 of the plasma, and there is no comment on alterations in acid-base equilibrium of his animals.

In the present experiments, the experiments of Woodbury were repeated. This procedure was shown to produce both hyponatremia and acidosis, and was accompanied by a significant decrease of the bone Na. This confirms the results of other workers,[9-14] indicating that hyponatremia and acidosis will cause a fall in the sodium of bone.

The mechanism of the acidosis produced by the Darrow Yannet experiment deserves some comment. Most simply viewed, there is a withdrawal of HCO_3^- from the body fluids into the petitoneum. In the Henderson-Hasselbach equation,

$$pH = pK + \log \frac{HCO_3^-}{H_2CO_3}$$

the numerator is lowered when the HCO_3^- moves into the peritoneal fluid, if no HCO_3^- is given to replace this loss, whereas the denominator of the equation will only be lowered temporarily because the respiratory mechanism maintains the level of H_2CO_3. This kind of metabolic acidosis might be considered analogous to the diarrheal acidosis in which HCO_3^- is lost from body fluids into the feces.

Nichols and Nichols[16] produced short term sodium depletion in dogs by intraperitoneal dialysis and found that the sodium content of a metatarsal bone before dialysis was greater than the sodium content after dialysis. From this data they concluded that the changes were caused by hyponatremia. However, it seems possible that their animals were not only hyponatremic, but also acidotic, since the total CO_2 of the plasma content at the end of the study was markedly lowered. Thus the decrease in bone sodium in the study of Nichols and Nichols could be due to acidosis or to hyponatremia, or to both.

Since infusion into the peritoneum of 5.5% glucose (10 c.c./100 grams body weight) results in both acidosis and hyponatremia, it had been hoped that, by infusing glucose and $NaHCO_3$ intraperitoneally, acidosis might be alleviated without preventing hyponatremia. These experiments did not succeed in maintaining the plasma CO_2 at the untreated level; however, though there is a statistical difference between the untreated group and the animals treated with glucose and $NaHCO_3$, it is doubtful if this difference is physiologically important.

Despite only minimal acidosis in this glucose and $NaHCO_3$ treated group of animals, bone sodium was significantly lowered (0.2534 meq./gram wet bone) by hyponatremia and Na depletion.

From these data it appears that hyponatremia, rather than acidosis, may be the important factor in decreasing bone sodium in the present experiments. This hypothesis is supported by the fact that in animals only negligibly acidotic, but moderately hyponatremic, there is as significant a lowering of the bone sodium as in animals which were more severely acidotic, and also moderately hyponatremic.

Further support for the theory that it is hyponatremia rather than acidosis which is the important factor in lowering the bone sodium can be obtained by applying the method of multiple regressions to the results of the experiments. Using this statistical method, the bone sodium can be expressed as a function of plasma sodium, and the acidosis is shown to be unimportant in determining the level of bone sodium in hyponatremia. The bone sodium may be estimated from the following equation:

$$\hat{Y} = 0.000674\ X_1 + 0.170274$$

where $\hat{Y}$ = the estimate of the bone Na and X_1 is the plasma sodium.

These results are in disagreement with the studies of Winters, Whitlock, DeWalt and Welt[19] whose data was interpreted to show that hyponatremia of relatively short duration is not associated with movement of bone Na. In the first part of their experiments using rats averaging 400 grams, Winters et al. produced hyponatremia with expansion of the body fluids, and found no change in bone Na. They did not measure plasma CO_2 in these animals. In another group of rats (average weight of 440 grams), they produced hyponatremia with a decrease in the extracellular fluid volume by peritoneal dialysis. The controls received 5% glucose in saline; the experimental animals received 5% glucose in water. Both groups of animals had a fall in their plasma CO_2 to 17 mM./L. Animals treated with glucose 5% in water had a fall of plasma Na; the data was interpreted as showing that with acidosis hyponatremia did not yield a change in bone Na. In a third group of animals, the experimental animals were hyponatremic and acidotic, and there was no change in the bone Na. Thus their conclusion that in hyponatremia of short duration there is not an associated fall in bone sodium.

Some workers[9] have described an increase in bone sodium with increase in age of the animal, the age of the animal being correlated with the gain in weight of the animal. In the present experiments it appears that for animals in the weight range 200 to 300 grams there was no significant difference in animals 250 grams and less as compared with animals more than 250 grams.

It appears likely that with increasing age, a progressively greater proportion of bone Na becomes inaccessible to rapid exchange with the fluids of the body.[20] One might predict that the sodium stores of the bones of young rats would prove more labile in response to Na depletion than would bone Na in old rats. Since the rats of Winter et al. were relatively old, weighing 380 grams to 460 grams, this may account for the discrepancy between their data and those of the present study. The results of the present study are in agreement with the reports of several other investigators,[9-14] who found that hyponatremia and acidosis produced a lowering in the sodium of bone.

From the experimental data it is possible to calculate the possible contribution of bone Na to the amount of depleted Na.

A 250 gram rat would receive 25 c.c. fluid intraperitoneally (10 c.c. per 100 grams body weight). Assuming that all of this fluid remained intraperitoneal during the experimental study, then the amount of Na lost to this fluid would be the product of the blood level of Na (since by four hours the intraperitoneal fluid and plasma would be in equilibrium) multiplied by the amount of fluid intraperitoneally or

25 c.c. intraperitoneally x .130 meq./c.c. = 3.3 meq. Na lost to intraperitoneal fluid

Another way to estimate Na depletion is by using the product of the plasma Na change times the total body water. Total body water is approximately 70% of body weight.

Change in [Na] in plasma x total body water

= 20 meq. Na x .70 x 250

= 3.5 meq. Na loss

The two calculations for total loss of Na correlate well.

It is possible to calculate the loss of Na from bone as follows:

Loss of bone Na is found from experimental data to be 0.01 to 0.02 meq./gram wet bone; since bone weight in rats is approximately 11% of body weight according to Shelton[21] (in dogs 17.4% of body weight and in man 15.9% of body weight) and approximately 10% according to Donaldson[20] then 10% x 250 grams = 25 grams of wet bone in 250 gram rats.

25 grams of wet bone x 0.01 to 0.02 = 0.25 to 0.50 meq. Na loss from bone

This 0.25 meq. to 0.50 meq. loss of Na from bone would replace 10 to 15% of the Na depleted from the total body fluids. It appears that the contribution of bone to the replacement of Na depletion is small, although it is known that bone contains one-half of the Na in the body.

SUMMARY

1. Rats infused with glucose intraperitoneally and exsanguinated four hours later had a significant depression of the plasma sodium, a moderately severe degree of acidosis, and a significant lowering of bone sodium as compared with untreated animals.

2. Animals infused with glucose and 25 meq./L. of $NaHCO_3$ and exsanguinated four hours later had a significant fall in the plasma sodium, a mild degree of acidosis, and a significant decrease in the bone sodium as compared with the untreated animals.

3. Comparing animals from 1 and 2 (above) it appeared that hyponatremia was the significant factor in producing the fall in bone sodium.

4. By the method of multiple regressions, in hyponatremia bone sodium can be expressed as a function of plasma sodium, and acidosis is shown to be unimportant in determining the level of bone sodium.

Estimated bone sodium $= \hat{Y} = 0.000674\ X_1 + 0.170274$

where X_1 is the plasma sodium.

TABLE I

Group A -- Untreated Animals

Plasma Na meq./L.	Plasma CO_2 mM./L.	Bone Na meq./g. wet bone	Weight
153	24.5	0.271	210
152	28.1	0.283	220
152	26.1	0.283	225
160	24.7	0.266	250
151	28.2	0.273	280
154	26.3	0.268	302
151	27.9	0.271	300
152	27.2	0.283	305
149	27.2	0.271	340
152.7	26.69	0.2743	
± 1.028	± 0.465	± .00227	

ZX
SE

Group B -- Animals treated with 5.5% glucose

Plasma Na meq./L.	Plasma CO_2 mM./L.	Bone Na meq./g. wet bone	Weight
136	22.7	0.240	190
135	22.3	0.276	220
137	20.3	0.264	225
134	20.2	0.264	240
132	19.5	0.269	280
118	14.4	0.263	290
128	19.3	0.264	290
137	18.1	0.275	310
132	19.1	0.256	300
129	18.8	0.265	310
131.8	19.47	0.2636	
± 1.83	± 0.729	± 0.00321	

Group C -- Animals treated with 5.5% glucose with 25 meq./L. $NaHCO_3$

Plasma Na meq./L.	Plasma CO_2 mM./L.	Bone Na meq./g. wet bone	Weight
129	26.6	0.246	230
125	25.5	0.265	237
128	22.4	0.246	240
126	24.4	0.241	245
132 / 128	25.1 / 24.80	0.269 / 0.2534	270
± 1.22	± 0.697	± 0.00566	

Group D -- Animals treated with 5.5% glucose with 25 meq./L. NaCl

Plasma Na meq./L.	Plasma CO_2 mM./L.	Bone Na meq./g. wet bone	Weight
124	23.5	0.248	220
134	23.2	0.246	235
132	15.2	0.238	250
135 / 131.3	24.1 / 21.5	0.264 / 0.2490	280
± 2.50	± 2.11	± 0.00545	

TABLE II

COMPARING UNTREATED ANIMALS WITH ANIMALS TREATED WITH 5.5% GLUCOSE

	PLASMA Na meq./L.	PLASMA CO_2 mM./L.	BONE Na meq./gram wet bone
UNTREATED ANIMALS 9 animals	152.7	26.69	0.2743
ANIMALS TREATED WITH GLUCOSE 10 animals	131.8	19.47	0.2636
	$p = < 0.001$ significant	$p = < 0.001$ significant	$p =$ between 0.05 and 0.01 significant

TABLE III

COMPARING UNTREATED RATS -- 250 grams and less with animals greater than 250 g.

	PLASMA Na meq./L.	BONE Na meq./gram wet bone	WEIGHTS
UNTREATED 250 grams and less four animals	154.3	0.2758	210 220 225 250 avg. 226
greater than 250 grams	151.4	0.2732	280 302 300 305 340 avg. 305
	p = greater than 0.05 not significant	p = greater than 0.05 not significant	

TABLE IV

COMPARING RATS TREATED WITH 5.5% GLUCOSE -- 250 grams and less with animals greater than 250 grams

	PLASMA Na	BONE Na	WEIGHTS
250 grams and less four animals	135.5	0.2610	190 220 225 240 avg. 219
greater than 250 grams six animals	129.3	0.2653	280 290 290 310 300 310 avg. 298
	p = greater than 0.05 not significant	p = greater than 0.05 not significant	

TABLE V

COMPARING ANIMALS TREATED WITH GLUCOSE AND $NaHCO_3$

vs ANIMALS UNTREATED
vs ANIMALS TREATED WITH GLUCOSE

	PLASMA Na	PLASMA CO_2	BONE Na
treated with glucose and $NaHCO_3$ five animals	128	24.80	0.2534
untreated nine animals	152.7	26.69	0.2743
	p = less than 0.001 significant	p between 0.05 and 0.01 probably significant	p between 0.01 and 0.001 significant
treated with glucose and $NaHCO_3$ five animals	128	24.80	0.2534
treated with glucose ten animals	131.8	19.47	0.2636
	p greater than 0.05 not significant statistically	p less than 0.001 significant	p greater than 0.05 not significant

TABLE VI

Data for multiple regressions number = 28

	PLASMA Na X_1	PLASMA CO_2 X_2	BONE Na Y
	153	24.5	0.271
	152	28.1	0.283
	152	26.1	0.283
	160	24.7	0.266
	151	28.2	0.273
	154	26.3	0.268
	151	27.9	0.271
	152	27.2	0.283
	149	27.2	0.271
	136	22.7	0.240
	135	22.3	0.276
	137	20.3	0.264
	134	20.2	0.264
	132	19.5	0.269
	118	14.4	0.263
	128	19.3	0.264
	137	18.1	0.275
	132	19.1	0.256
	129	18.8	0.265
	129	26.6	0.246
	125	25.5	0.265
	128	22.4	0.246
	126	24.4	0.241
	132	25.1	0.269
	124	23.5	0.248
	134	23.2	0.246
	132	15.2	0.238
	135	24.1	0.264
ZX	3857	644.9	7.368
ZX	534783	15252.13	1.943538

Y estimated =

$$\hat{Y} = 0.000702\ X_1 - 0.0000134\ X_2 + 0.166749$$

testing significance of two regressions

$b'_{y1.2} = 0.604101$

$b'_{y2.1} = 0.038946$

Explanation of Table VI (cont'd)

significance of $b'_{y1.2} = t = \frac{0.604101}{0.206363} = 2.93$

p is less than 0.01 and thus is significant

significance of $b'_{y2.1} = t = \frac{-0.038946}{0.206363} = 0.081$

p is greater than 0.5 not significant

thus

$$\hat{Y} = 0.000674 \quad X_1 + 0.170274$$

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